Current Biology Vol 22 No 3
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Plant Self-Incompatibility: Ancient System Becomes a New Tool

Expressing a pollen self-incompatibility gene from *Papaver rhoeas* (poppy) in *Arabidopsis thaliana* renders the latter sensitive to an exquisitely precise induced cell death response. This simple system may have wide application in biotechnology and research.

Bruce McClure

Many wild plants possess efficient mechanisms for ensuring outcrossing, but these mechanisms have often been lost in domesticated crops. Much progress has been made toward understanding the mechanisms plants use to control mating, but until now it seemed that the prospects for transferring these controls across wide phylogenetic distances were not good. Recently published in *Current Biology,* de Graaf *et al.* [1] show that such a transfer may be easier than previously thought. Self-incompatibility (SI) systems are genetically controlled mechanisms that favor outcrossing in many wild plant species. SI species maintain generally high levels of hybrid vigor. In particular, heterozygosity at the controlling *S*-locus is enforced by the SI mechanism itself — in *Papaver rhoeas*, for example, haploid pollen is rejected whenever its *S*-haplotype is the same as either of the two *S*-haplotypes expressed in the diploid female tissues. The specificity implied by this type of pollen–pistil recognition is extraordinary, especially when considered at the population level. For example, while *P. rhoeas* populations of just a few dozen individuals may contain 25 to 30 distinct *S*-haplotypes, the species as a whole has many more [2,3].

This diversity at the genetic level is reflected at the biochemical level in highly specific interactions between pollen and pistil proteins encoded by *S*-haplotype-specific genes. The *P. rhoeas* genes expressed in the pistil and in pollen are referred to as *PrsS* and *PrpS,* respectively (e.g., the S_1-haplotype encodes $PrsS_1$ and $PrpS_1$ proteins). PrsS proteins are secreted onto the stigma surface, where they define mating type on the female side [4]. PrpS proteins are located in the pollen tube membrane and similarly define *S*-specificity on the male side [5]. Interactions between these proteins are sufficiently specific to allow mate selection even in a highly diverse population. A single PrpS protein must recognize as 'self' only one of the 25 to 30 PrsS proteins it might encounter in the population to initiate the rejection response.

Physiological studies have revealed a staged, multilayered SI mechanism that culminates in a programmed cell death response in incompatible pollen tubes. *P. rhoeas* pollen tubes immediately dissipate their steep tip-focused calcium gradient and cease growth upon exposure to a self-PrpS protein [6,7]. Rapid depolymerization of the actin cytoskeleton is accompanied by several metabolic changes [8,9]. A longer-term, irreversible rejection response probably begins with actin depolymerization and increased mitogen activated protein kinase (MAPK) activity observable after 10 minutes [10]. Features of programmed cell death (e.g., mitochondrial cytochrome c release, activation of a caspase-like protease, and DNA fragmentation) are observable over timescales of 1 to 4 hours [11,12]. Importantly, this orchestrated sequence of responses can be initiated by self-PrsS protein expressed in bacteria, a clear indication that no other stigmatic factors are required. Nonself-PrsS proteins have no effect. The *PrsP* gene was identified by close linkage to *PrsS* genes, polymorphisms consistent with the genetic diversity of SI, and an *S*-specific inhibition of pollen tube responses when *PrsP* gene expression is inhibited [5].

Recently, de Graaf *et al.* [1] report that expression of *PrsP* in pollen of self-compatible *Arabidopsis thaliana*, a species whose ancestors diverged from the *Papaver* lineage about 140 Mya, initiates a response similar to that seen in *Papaver* when exposed to PrsS. The authors used a pollen-specific promoter from tobacco to drive expression of a *P. rhoeas* $PrsP_1$:GFP fusion in *A. thaliana* pollen. They elegantly show that exposing pollen from a plant hemizygous for the *PrsP1:GFP* transgene to $PrsS_1$ protein reduces pollen viability by half; significantly, only the GFP pollen is affected. The gold standard for testing any specific pollination response is to determine the effects of allelic *S*-proteins. Thus, the authors created two *A. thaliana* lines homozygous for either a $PrsP_1$ or $PrsP_3$ construct and tested each for its response to $PrsS_1$ and $PrsS_3$ proteins. Exactly as expected, each line is inhibited by only the self-PrsS protein. Nonself-protein or denatured protein shows no effect. The authors provide multiple lines of evidence to show that *A. thaliana* pollen inhibition is similar to that seen in *P. rhoeas*. Visualization of the actin network in *A. thaliana* pollen expressing $PrsP_1$ or $PrsP_3$ and exposed to self-PrsS protein show dramatic shifts in the structure of the cytoskeleton, a hallmark feature of the early response in *P. rhoeas*. A caspase-3 inhibitor was used to test whether transformed pollen displayed an activated programmed cell death pathway. The results of this experiment show that this inhibitor mitigates the loss of viability caused by self-PrsS proteins to a similar extent in both *P. rhoeas* pollen and in the transformed *A. thaliana* pollen.

SI genes have previously been transferred to *A. thaliana* from its close SI relative *Arabidopsis lyrata* [13]. *A. lyrata* SI genes work in *A. thaliana* precisely because the species are so closely related. SI was lost in the *A. thaliana* lineage relatively recently, and different accessions show different defects in SRK-SCR genes [14]. SI in Brassicaceae differs in almost every imaginable respect from SI in *Papaver*, apart from the fact that *S*-specificity is determined by pairs of tightly linked pollen- and pistil-specific genes. In *Papaver*, the physiological action of the SI mechanism is on the pollen side — the PrsS protein ligand is expressed in the pistil and triggers responses in self-pollen. In contrast, SI *A. lyrata* and other Brassicaceae display sporophytic SI; the pollen *S*-determinants (SCR) are present on the pollen grain surface, and stigmatic papillar cells express *S*-haplotype-specific receptor

kinases (SRKs), [15]. Here, it is the pollen protein that acts as a ligand and triggers a response in the pistil that determines whether pistil resources are made available for pollen hydration, germination, and tube growth. Transferring SI genes from *A. lyrata* to *A. thaliana* works because the *A. lyrata* SRK proteins can interact productively with *A. thaliana* signal transduction proteins and elicit an appropriate response. Transfer of SI from *A. lyrata* to *A. thaliana* makes it possible to use the genetic tools available in the latter to dissect SI [16]; these tools will now be helpful for testing hypotheses about SI in *Papaver*. However, it may be possible to do far more.

The phylogenetic distance between *P. rhoeas* and *A. thaliana* is great, and yet an SI response can be triggered. This finding suggests that the *Papaver* SI system must be accessing a highly conserved set of responses. de Graaf *et al.* [1] suggest that it may be possible to use the *P. rhoeas* system to introduce a controlled pollination system into a wide variety of plants. This control has long been a goal of plant biotechnology. The advantages of hybrid crops like maize and rice are well known, but producing the hybrid seed is expensive. Transgenic approaches for making hybrids have been developed, but none have found wide application [17] — in part, because they rely on systems for making one parent of the hybrid sterile, which causes inherent difficulties. SI systems in wild plants, like *P. rhoeas*, provide the benefit of hybrid vigor, but all the plants are fertile and outcrossing is favored by controlling which pollen is accepted or rejected. Thus, the specificity of the *P. rhoeas* system and its apparent ability to work across great phylogenetic distances may make it well suited for engineering hybridity in crops. But why stop there? The experiments by de Graaf *et al.* [1] demonstrate an inducible cell death system with exquisite specificity, a potentially very useful research tool. For example, *PrpS* genes or multiple allelic *PrpS* genes might be used in cell fate studies to test the effects of removing specific cell populations by expressing the genes with cell-type-specific promoters and treating with the appropriate incompatible PrsS protein. Will the system work across even greater phylogenetic distances? Time will tell what applications will ensue.

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NSF disclaimer: The author is temporarily working at the US National Science Foundation. The views and opinions expressed in this article are those of the author and do not necessarily represent the views or policies of the National Science Foundation.

DOI: 10.1016/j.cub.2011.12.034

Calcium Signaling: Deciphering the Calcium–NFAT Pathway

Rapid cellular calcium oscillations activate gene expression hours later. How this temporal response amplification is achieved has until now been largely a mystery. An elegant combination of experimental strategies and a model that encompasses non-linear inputs and outputs now sheds new light on this long-standing problem.

James W. Putney

At some point in their growth, differentiation or function, every cell in the body is affected by calcium signaling, a process whereby external signals interact with cells to cause their cytoplasmic Ca^{2+} to rise. This rise in cytoplasmic Ca^{2+} triggers cellular responses over time courses that range from subsecond (neurotransmitter release for example) to hours or days (gene regulation and differentiation). In this issue of *Current Biology*, Kar *et al.* [1] describe a new and intriguing level of complexity in the process by which Ca^{2+} signals regulate gene expression.